A BEHAVIORAL MECHANISM FOR OSMOTIC REGULATION IN A SEMI-TERRESTRIAL CRAB

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It is generally known that an animal can regulate osmotically by possessing a relatively impermeable integument and by utilizing specialized organs which by active metabolic processes control the flux of salts and water between animal and its external medium. However, there is evidence that animals are capable of assisting the osmo-regulatory mechanism by their behavior.

Barnes (1935, 1938, 1940) demonstrated that the isopod, *Ligia baudiniana*, prefers distilled water to normal sea water when the choice is offered in the form of moistened filter paper. Gross (1955) revealed that the land crab, *Birgus latro*, can control the osmotic pressure of its body fluids by selection of appropriate sea water concentrations. Krijgsman and Krijgsman (1954) have produced evidence of an osmo-receptor in the spiny lobster *Jasus* which apparently serves to guide the animal with respect to salinities.

Pachygrapsus crassipes is a crab of semi-terrestrial habits, most often found in exposed, rocky shore situations, but also occasionally found in protected bays in muddy burrows. Typically, it does not inhabit waters deviating much in salinity from normal sea water, but it can regulate osmotically in both dilute and concentrated sea water (Jones, 1941; Gross, 1955; Prosser *et al.*, 1955). Gross (1955) raised the question as to the adaptive significance of strong osmo-regulatory powers in this crab which is extremely agile and easily capable of reaching normal sea water should it by chance venture from the sea into an osmotic stress such as would be afforded by an isolated, evaporated tide pool, or one diluted by rain water.

In Southern California, situations affording such osmotic stresses are rare. However, there undoubtedly are regions in this crab's range where osmotic stresses are readily enough available so that selection would favor the development of an osmo-regulatory mechanism. On the other hand, should *Pachygrapsus* be capable of detecting salinities deviating from normal sea water and should it shun abnormal salinities, then the adaptive significance of osmotic regulation in this species could remain in doubt.

The present investigation will show that *Pachygrapsus* does show preference for sea water of normal salinity.

Materials and Methods

Crabs of the species *Pachygrapsus crassipes*, collected at Laguna and Newport, California, were placed singly in a box in which there were four containers, each containing sea water of a different salinity. The containers were placed in each corner of the box and sunk so that their rims were flush with the platform which

constituted a second floor of the box. In each container was a treadle which could be depressed by about 15 grams. The surface of the treadle was barely below the water level in the container.

As a crab entered a given medium, it was necessary for it to depress the treadle. This caused the deflection of a signal magnet which was recorded on a kymograph drum, rotating at a known rate. Thus a crab placed in the box had the choice of remaining on the platform in the air or entering any of the containers in each of the four corners of the box. The kymograph record then would indicate the movements of the crab while it remained in the box, what container it entered, when it entered and how long it remained.

The selection box was placed in a darkened, quiet, temperature-controlled room (20° C.). As a control against selection for any given corner, rather than salinity, the respective sea water concentrations were rotated in position with each successive test specimen. In most cases only three containers were filled with water; the fourth remained empty to check on the preference for corners. Each crab recorded, remained in the box for at least 40 hours.

Sodium and potassium concentrations of the blood were determined in a few cases by means of a Beckman flame photometer.

Results

Table I summarizes the results of the following experiments:

1. Crabs freshly removed from normal sea water were given a choice of 50, 100, or 150% sea water (seven crabs).

Table I

Salinity preference in Pachygrapsus crassipes

Treatment	Mean time spent in selected salinities										Time spent out of water (%)
	50% sea water		75% sea water		100% sea water		125% sea water		150% sea water		
	% time	time (hr.) / No. visits	% time	time (hr.) / No. visits	% time	time (hr.) / No. visits	% time	time (hr.) / No. visits	% time	time (hr.) / No. visits	
Normal (7 animals)	6.2	3 / 32			29.7	16 / 55			3.7	2 / 29	59.7
Normal (5 animals)			6.1	3 / 12	39.6	17 / 49	6.9	3 / 24			47.5
Acclimated to 50% sea water (5 animals)	7.7	4 / 42			29.8	15 / 37			7.3	4 / 33	55.4
Acclimated to 150% sea water (5 animals)	13.5	7 / 32			16.1	9 / 22			10.1	6 / 21	60.3
Desiccated (6 animals)	11.4	8 / 51			47.6	29 / 54			3.6	2 / 32	37.5

Legend: Normal = animals freshly removed from normal sea water;
% time = % total time spent in chamber.

2. Crabs freshly removed from normal sea water were given a choice of 75, 100 or 125% sea water (five crabs).

3. Crabs acclimatized to 50% sea water 2–7 days were given a choice of 50, 100 or 150% sea water (five crabs).

4. Crabs acclimatized to 150% sea water 1–3 days were given a choice of 50, 100 or 150% sea water (five crabs).

5. Crabs desiccated 2–4 days were given a choice of 50, 100 or 150% sea water (six crabs).

Of the above 28 specimens studied in the selection chamber, 25 spent more time in 100% sea water than any salinity offered, thus indicating that *Pachygrapsus* does show a decided preference for normal sea water. Two of the above exceptions were among the group which had been acclimated to 150% sea water. The third was one of those which had been desiccated. Although Table I shows that animals first acclimatized to 150% sea water average more time in 100% sea water than any other salinity, the histories of the individuals do not show such a preference.

All six of the crabs which were desiccated before being placed in the box remained in water longer than in the air, which might be expected. However, of the remaining 22 test crabs, 17 spent more time out of the water than in all salinities offered, thus suggesting the degree of the aerial habit in this marine form.

Considering individual histories, 14 crabs spent more time in the hypotonic medium than in the hypertonic medium. Six spent more time in the concentrated media and eight showed no significant preference between the two types of stresses. It is interesting, however, that of the 14 preferring dilute to concentrated media, four had been previously acclimated to 150% sea water, only one of which preferred dilute to normal sea water. Three of the above 14 had been desiccated previously, one of which showed a preference for dilute sea water over normal sea water. With the sample at hand, nothing can be said concerning the preference of dilute and concentrated sea water or vice versa.

In only one of the 24 cases where one of the four containers was not filled with water did a crab spend more time in the empty container than it did on the platform between the containers, *i.e.*, out of the water. Thus, there does not seem to be any particular preference for corners, at least in an aerial situation.

Of 25 crabs showing preference for 100% sea water, judging by the time they spent in this salinity, 14 made more visits to normal sea water than any other offered aqueous medium. On the other hand, 11 made more or an equal number of visits to other than 100% sea water. Thus, with the sample at hand, nothing can be resolved precisely as to the learning process involved.

Table II shows the blood sodium and potassium concentrations of some of the crabs which had spent at least 40 hours in the selectivity chamber. Here it can be seen that the blood sodium of one of the five crabs which was first acclimated to 150% sea water (number 4) was definitely above the normal concentration. It should be pointed out, however, that this particular specimen was somewhat weakened by its previous treatment in 150% sea water and this was the only crab that spent more time in concentrated sea water than in any of the other salinities. Thus, four out of five specimens in the group attained close to normal blood concentrations after a period in the selectivity chamber. The blood sodium concentration of two out of the three of the desiccated group sampled was below the normal concentration range. This is particularly interesting in the case of specimen num-

TABLE II

Blood sodium and potassium concentrations in Pachygrapsus following period in selection chamber

Specimen No.	Treatment	Blood concentration after treatment*	
		Na (meq/l)	K (meq/l)
1	Normal	484	6.35
2	Acclimatized to 50% sea water	498	—
3		481	8.08
4	Acclimatized to 150% sea water	544	7.72
5		477	6.62
6		477	7.66
7		495	8.81
8		498	8.06
9	Desiccated	417	7.35
10		479	8.50
11		414	10.4
95% fiducial limits**		478–489	7.19–7.67

* Concentration choices available for all specimens were 50, 100, and 150%.
** Calculated from observations on 36 crabs freshly removed from the sea.

ber 9 which spent 51% of its time in 100% sea water while it was in the chamber. Specimen number 11, however, spent more time in 50% sea water than in any other offered salinity. The remaining cases shown in Table II are within or close to the normal blood concentrations with respect to sodium.

Considering the intrinsic error in determining potassium concentrations by the methods used in this investigation (about 10% of the concentration of normal blood), only the blood potassium of specimen 11 (Table II) seems to vary from normal. This, however, would have little effect on the osmotic pressure of the blood.

The activity in the selection chamber of the above crabs also was considered with respect to the time of day and phase of the tide, but no periodicity could be resolved.

DISCUSSION

Using duration of immersion as a criterion, *Pachygrapsus* prefers normal sea water to hypotonic or hypertonic media varying at least 25% from 100% sea water. It thus seems that in a natural situation, this crab probably would not remain long in an osmotic stress, but soon would return to the salinities of normal

sea water. *Pachygrapsus* possesses a relatively impermeable integument (Gross, 1957) and this means that there would be a strong passive resistance to salt-water exchanges, should the crab enter an osmotic stress. The question is posed then as to the need for osmotic regulation in an animal which is rarely exposed to such stresses and which would attempt to escape, and could escape to the comfort of the nearby sea should the occasion arise.

The strong preference for normal sea water, it would seem, would be a powerful factor tending to restrict this species to the intertidal and subtidal zones of the sea. For example, a period of desiccation, such as might be encountered by remaining out of the water, would stimulate the return to the water, but as suggested above, not just to any water, but to normal sea water. Again, in an estuarine situation, where a sharp salinity gradient would be available, an animal responding thus to osmotic stresses would tend to avoid brackish water, even though it possessed the ability to regulate strongly in such stresses. Even in a case where a population were temporarily trapped in a dilute situation for an extended period, this period of acclimation, as shown above (Table I), does not seem to diminish the preference for normal sea water.

Of course, the distaste for brackish water possibly could be overcome by other factors, *e.g.*, food source and retreat from predators.

Acclimation to 150% sea water apparently breaks down the preference for normal sea water (Table I), although the fact that four out of five of the specimens thus treated were able to achieve normal blood concentrations suggests effective salinity selection of a sort. This preference breakdown perhaps is correlated with the ability of *Pachygrapsus* to regulate more strongly in dilute than in concentrated media (Jones, 1941; Gross, 1955; Prosser *et al.*, 1955). In such case, a period of acclimation would necessitate a greater alteration on the physiological condition of the organism and consequently a greater effect on its behavior. Then, too, a precise "knowledge" of external salinities possibly is necessary for adequate regulation. Should a period of immersion in 150% sea water reduce the accuracy of the appropriate receptors, whatever they may be, and efficiency of such receptors were necessary for regulation, then it would follow that *Pachygrapsus* would show relatively weak regulation in the concentrated sea water.

On the other hand, as shown above, acclimation to 50% sea water does not appreciably affect the preference for normal sea water. This may be simply because *Pachygrapsus* can regulate strongly in dilute media and the consequent physiological alteration in the crab would be held at a minimum. Or the hypotonic media may not reduce the efficiency of the osmo-receptors and the organism, being "aware" of the external salinity, could regulate accordingly.

It seems, then, that should a population of *Pachygrapsus* be confined to a hypertonic medium for an extended period, by losing its preference for normal sea water, it might remain in such concentrated environments, assuming all other biological requirements were satisfied. However, it is difficult to imagine natural hypertonic situations capable of isolating *Pachygrapsus* from the sea.

Table II demonstrates the general tendency for *Pachygrapsus* to achieve normal blood sodium concentrations after a period in the selectivity chamber. This seems to be true even when blood concentrations were forced away from normal by acclimation to 50% and 150% sea water. Two exceptions, however, were crabs

previously desiccated. The final blood concentrations in these cases were lower than normal, thus suggesting over-compensation.

It has been demonstrated numerous times that organisms immersed in stress media metabolize more rapidly than when they are in their normal media (Schlieper, 1929; Schwabe, 1933; Flemister and Flemister, 1951). This increased metabolism has been interpreted often as added osmotic work. However, doubts have been thrown on this interpretation (Krogh, 1939; Wikgren, 1953; Potts, 1954).

Gross (1957) reports that crabs immersed in stress media apparently attempt to escape and thus become more active. He suggested, then, that increased oxygen consumption in increased osmotic stresses was merely the reflection of the attempt to escape an uncomfortable medium. The preference for normal sea water established quantitatively by the present investigation corroborates this suggestion.

With the exception of those crabs first desiccated, the experimental animals spent about half their time out of water. This suggests a high degree of adaptation to the aerial habit, but not so much compared with the land crab *Birgus* which under experimental conditions spends only about one hour per day visiting water (Gross, 1955).

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Summary

1. The shore crab *Pachygrapsus crassipes* prefers 100% sea water to 50, 75, 125 and 150% sea water.

2. This preference could not be altered by first desiccating the animal or by acclimating the animal for several days in 50% sea water. The preference could be altered somewhat by acclimating to 150% sea water.

3. The preference for normal sea water by *Pachygrapsus* suggests a mechanism which tends to restrict this crab to the intertidal and subtidal zones of the sea.

4. *Pachygrapsus* under the experimental conditions of the present investigation spends about 12 hours per day visiting water. This compares to one hour per day for the land crab *Birgus*.

5. *Pachygrapsus* tends to maintain normal sodium and potassium blood concentrations when given free choice of salinities, including 100% sea water. Normal concentrations are generally achieved under the same conditions even when the blood has been forced away from normal by acclimation in 50 or 150% sea water. However, animals previously desiccated may over-compensate when offered a choice of media varying in salinity and consequently achieve blood sodium concentrations below the normal range.

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